Effect of Elevated Growth Hormone Concentrations on the Phenotype and Functions of Human Lymphocytes and Natural Killer Cells

Abstract
Over the past decades, strong evidence has accumulated that growth hormone (GH) has immunostimulatory properties. Therefore, an investigation was conducted on 10 acromegalic patients and 9 age- and sex-matched healthy controls to determine whether plasma GH concentrations correlate with changes in several immune parameters, including serum concentrations of immunoglobulins, lymphocyte subsets, lymphocyte transformation with phytohemagglutinin (PHA), natural killer (NK) cell activity as well as phagocytic and metabolic burst activity. While NK cell activity, serum concentrations of immunoglobulins (IgG, IgM, IgA) and metabolic burst activity were within the normal range in both groups, a significantly enhanced phagocytic activity was observed in the acromegalic patients. Surface markers on T lymphocytes (CD3, CD4, CD8), B lymphocytes (CD19) and NK cells (CD16/56) were normal in both groups; however, in the acromegalic subjects, CD4+ and CD8+ cells showed a significant higher expression of transferrin receptors (CD71), indicating enhanced T-cell activity. The lymphocyte transformation response to PHA at the highest concentration tested showed a tendency to be elevated in acromegalics; however, the difference failed to be significant. Long-lasting and pronounced elevation of GH in acromegaly induces significantly enhanced phagocytic activity, but only negligible changes in most patients in lymphocyte phenotype and in the lymphocyte response to PHA.

Introduction
It has been postulated that growth hormone (GH), a 22-kD protein synthesized by the adenohypophysis, has immunostimulatory properties [1, 2]. Studies in hypophysectomized mice and GH-deficient patients have revealed several alterations in the immune system, such as reduced activity of natural killer (NK) cells, thymic atrophy, reduced antibody synthesis and a significant delay in rejecting allogenic skin grafts [3–7]. On the other hand,
increased activities of cytotoxic T lymphocytes and NK cells have been observed in vitro when lymphoid cells are incubated with GH [8] and in vivo after GH substitution in GH-deficient patients and mice [9, 10]. Additionally, the phagocytic activity expressed as the production of O₂ by zymosan-stimulated macrophages was identical in a group of hypophysectomized rats treated either with GH or recombinant rat interferon-γ (IFN-γ), the classic macrophage-activating factor [11]. Furthermore, granulocytes from patients with acromegaly have a significantly higher resting and starch-elicited metabolic burst activity [12]. However, in one study in acromegalic patients where an increased bactericidal activity of monocytes against Mycobacterium avium was seen, no significant influence of GH on various components of cellular immunity could be detected [13].

The aim of this study was to determine whether chronically elevated plasma GH concentrations would be associated with alterations to the immune system. For this purpose, several parameters of the humoral and cellular immune system including NK cell activity, T- and B-lymphocyte numbers, lymphocyte subsets, lymphocyte transformation with phytohemagglutinin (PHA), the phagocytic activity and the oxidative burst of leukocytes were determined in acromegalic patients and healthy controls.

### Materials and Methods

#### Patients

Ten patients with active acromegaly (mean age 50.9, range 33–68 years) and 9 sex- and age-matched controls were studied after informed consent was obtained. Characteristics of the patients are shown in table 1. The mean plasma GH concentrations of the acromegalic patients at the time of investigation was 51.7 ± 24.2 μg/l, the mean insulin-like growth factor I (IGF-I) concentration was 3.6 ± 0.7 U/ml (table 2). The GH levels of the healthy controls were within the normal range (table 3). Serum prolactin concentrations were normal in all patients and control subjects (tables 2, 3). Six patients had been previously operated upon and 5 patients had been taking drugs (3 patients a somatostatin analogue, 2 patients a dopamine agonist), which were stopped 2 weeks before blood was taken. The investigation was performed at 08:00 h after an overnight fast and in the previous 2 weeks there had been no history of infections or surgery, which may alter the immune system. One patient with secondary hypothyroidism and adrenal deficiency received adequate substitution therapy (100 μg L-thyroxine, 10 mg hydrocortisone; additionally, 3 postmenopausal women were substituted with estrogen along with progestin). Immunoglobulin measurements were quantified by radiodiffusion [14].

#### Immunofluorescence and Flow Cytometry

100-μl aliquots of heparinized whole blood were stained with different anti-CD monoclonal antibodies (mAbs) for 20 min at room temperature (RT). Red blood cells were then lysed in 1 ml FACSDying solution (purchased from Becton Dickinson, San Jose, Calif., USA), containing <50% diethylene glycol and <15% formaldehyde, for 15 min at RT then pelleted at 400 g for 6 min. Following one washing step, 1.5 x 10⁴ cells were analyzed on a flow cytometer (FACScan; Becton Dickinson). For two-color analysis, cells were stained with fluorescein isothiocyanate (FITC)- and PE-labeled anti-CD45 vs. CD14, anti-CD3 vs. CD19, anti-CD3 vs. CD4, anti-CD3 vs. CD8 and anti-CD3 vs. CD16/56 (Simultest IMK plus reagent kit; Becton Dickinson), respectively. Three-color staining of CD4, CD8

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**Table 1.** Patients’ characteristics of the acromegalic disease treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
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<th>Replacement therapy</th>
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</tr>
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</tr>
<tr>
<td>9</td>
<td>M</td>
<td>75</td>
<td>3</td>
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<td>M</td>
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DA = Dopamine agonist; S = somatostatin analogue.
Table 2. Biochemical parameters of endocrine status in acromegalic patients

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<th>Patient</th>
<th>Sex</th>
<th>GH µg/l</th>
<th>SMC U/ml</th>
<th>T₄ ng/ml</th>
<th>T ng/dl</th>
<th>Estradiol pg/ml</th>
<th>PRL µg/l</th>
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Normal 0-5 0.3-1.9 50-120 200-800 0-55 post- and 50-400 pre-menopause

Table 3. Patient characteristics and endocrine status of the controls

<table>
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<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>GH µg/l</th>
<th>T₄ ng/ml</th>
<th>T ng/dl</th>
<th>Estradiol pg/ml</th>
<th>PRL µg/l</th>
<th>Replacement therapy</th>
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<td>conjugated estrogen + medrogeston</td>
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</tbody>
</table>

Normal 0-5 50-120 200-800 0-55 post- and 50-400 pre-menopause

and CD19 subtypes was performed with anti-CD45RA, CD45RO, HLA-DR, CD25 (IL-2 receptor), CD28, CD69 (all purchased from Becton Dickinson), CD71 (transferrin receptor) (Immunotech, Marseille, France). Lymphocytes were identified by light scatter characteristics (cell size and granularity) and CD45 expression. Isotype-matched mouse antibodies (IgG1 and IgG2) conjugated with FITC, PE or PerCP (Becton Dickinson) were used as negative controls.

Determination of NK Cell Activity

Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation of whole heparinized venous blood over a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient. PBMC were subsequently suspended in RPMI 1640 (Gibco, Paisly, UK) supplemented with 100 IU penicillin and 100 µg streptomycin (Gibco) per milliliter and adjusted to 1 x 10⁶ PBMC/ml. NK assays were performed as described previously [15]. Each experiment was carried out with an at least equal number of control persons as patients.

Three effector:target (E:T) ratios (100:1, 50:1, 25:1) were prepared, in which ⁵¹Cr-labeled (Behringwerke AG, Marburg, Germany) NK-sensitive K562 cells served as targets and PBMC were used as effector cells.

Target cells (0.125 x 10⁶/100 µl) were added to 100 µl effector cells in the appropriate dilution. All assays were performed in triplicate for each patient and each E:T cell ratio in serum-free RPMI 1640 supplemented with 100 IU penicillin and 100 µl streptomycin/ml. Suspensions of effector and ⁵¹Cr-labeled target cells had been centrifuged at 100 g at RT for 3 min, and incubated for 4 h at 37°C in a humidified atmosphere containing 5% CO₂. Radioactivity in the supernatant was measured in a gamma counter (Becton Dickinson). The percentage of specific lysis was assessed according to the formula:

% specific lysis = \( \frac{\text{Experimental lysis} - \text{Spontaneous lysis}}{\text{Maximum lysis} - \text{Spontaneous lysis}} \times 100. \)

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Kotzmann/Köller/Czemin/Clodi/Svoboda/Riedl/Boltz-Nitulescu/Zielinski/Luger

Effect of Elevated Growth Hormone on the Immune System
Maximum lysis was determined by treating $^{31}$Cr-labeled K562 cells with 1% SDS in distilled water.

Phagocytosis of opsonized and FITC-conjugated *Escherichia coli* and metabolic burst were determined with Phago Test and Burst Test kits (Orpegon, Heidelberg, Germany), respectively, according to the manufacture's instructions, and analyzed by flow cytometry.

**Mitogen-Induced Lymphocyte Proliferation**

PBMC were isolated by density gradient centrifugation with Ficoll-Paque (Pharmacia). Autologous serum was collected before removing the separated cells from Ficoll. After two washing steps, cells were resuspended in RPMI 1640 medium (Sigma, St. Louis, Mo., USA) containing penicillin (50 U/l) and streptomycin (50 µg/ml), both from IRH Biosciences (Lenexa, Kans., USA), and 25% autologous serum. PBMC were seeded on a 96-well microtiter plate (10^5 cells/well) with different PHA concentrations (12.5, 6.25, 3.12, 1.56, 0.75, 0.37 µg/ml) and incubated for 48 h at 37°C in a fully humidified air atmosphere containing 5% CO₂. Controls contained only medium. The cells were pulsed with $^3$H-thymidine (1 µCi/well) for a further 18 h. Cells were then recovered with a cell harvester (1295-001 Cell Harvester, LKB Wallac, Turku, Finland). Incorporated activity was measured with a Betaplate Liquid Scintillation Counter (1205 Betaplate TM; LKB Wallac).

**Statistical Analysis**

Data are expressed as the mean ± SEM. Means were compared by the Wilcoxon signed rank test for unpaired data and the differences were considered significant with p < 0.05.

**Results**

**Immunoglobulins**

When serum immunoglobulin (IgG, IgM, IgA) levels were measured, they were found to be within the normal range and did not significantly differ between the two groups (table 4).

**Cell Phenotype**

The distribution of cell surface markers of T lymphocytes (including CD3+, CD4+, CD8+), B lymphocytes (CD19+) and NK cells (CD16/56+) as well as the CD4/CD8 ratio were evaluated and found to be within the normal range and did not differ significantly from the control group (fig. 1). No significant differences in the expression of CD45RA, CD45RO, HLA-DR, CD25, CD28, CD69 were found, but in contrast, expression of CD71 (transferrin receptor) was higher in all lymphocyte subsets in the acromegalic subjects, but the difference reached significance only in the CD4+ (27.5 ± 4% vs. 13.7 ± 3.8%, p < 0.05) and CD8+ cells (19 ± 2.2% vs. 7.1 ± 3.5%, p < 0.05), when compared to healthy controls (fig. 2-4).

**NK Cell Activity**

In comparison with normal controls, no significant differences in NK cell activity were observed in the 10 acromegalic patients. The lytic activity was similar at different E:T ratios (fig. 5). Thus, no correlation between plasma levels of GH or IGF-I on one hand and NK activity on the other hand could be detected.

**Phagocytosis and Metabolic Burst**

When the formyl-methionine-leucine-phenylalanine (fMLP)- and *E. coli*-induced metabolic burst of leukocytes were investigated, they were also found to be within the normal range in all acromegalic patients (fig. 6b), but the percentage of phagocytizing granulocytes when incubated with FITC-labeled opsonized *E. coli* was significantly higher in the patients with acromegaly than in the control group (89 ± 3.4% vs. 51.1 ± 13%, p < 0.05) (fig. 6a).

**Mitogen-Induced Cell Proliferation**

Although the lymphocyte response to PHA in lower concentrations was comparable to the control group, we found in the acromegalic patients a tendency to increased lymphocyte mitogenesis at higher PHA concentrations, which failed to be significant (fig. 7).
Fig. 2-4. Expression of different activation markers on (2) CD4+, (3) CD8+, (4) CD19+ lymphocytes. Data from 9 healthy controls and 10 acromegalics are presented as mean ± SEM.

Discussion

Studies in hypophysectomized mice and GH-deficient patients, as well as in vitro studies on the effect of GH on lymphocytes, have suggested that the immune system of patients with acromegaly might develop considerable alterations [3-10]. The detection of specific receptors for GH and IGF-I on immunocompetent cells provided further evidence for a link between the pituitary gland and the immune system [16-18].

The activity of NK cells in patients with active acromegaly was found to be normal when compared to a control group in the present study. The increased incidence of neoplastic diseases observed in acromegalic patients [19-21] thus might be due to the ability of GH to either directly induce neoplastic changes in various organs or to enhance cell proliferation and cell transformation as described for normal and leukemic human lymphocytes [22]. In addition, GH induces proto-oncogenes such as c-myc [23].

Previous studies in GH-deficient children have revealed a normal or slightly reduced number of NK cells, expressing CD16 antigens [16], whereas the activity of these cells was found to be significantly lower when compared to a control group [24, 25]. This diminished NK activity could be partially restored in GH-deficient patients and hypophysectomized mice when their defect was treated with GH [9, 10]. An additional functional defect in NK cells has been postulated, because NK cells also failed to increase their cytotoxic activity in response to IFN-γ treatment [24]. However, acromegalics had comparable NK cell number and NK cell activity to normal controls, which is in agreement with a previous study [13]. In addition to GH, sex steroid hormones could also affect the percentage of CD16+ and CD4+ cells. Thus a possible stimulatory effect of GH on NK cell number or activity could have been masked by sex steroid deficiency. Testos-
Fig. 6. a Percent of phagocytizing granulocytes after incubation with FITC-labeled opsonized E. coli. b Percent of oxygen radical producing granulocytes incubated with fMLP or opsonized E. coli. Data from 9 healthy controls and 10 acromegalics are presented as mean ± SEM.

Fig. 7. Lymphocytes were cultured with different concentrations of PHA and proliferation was determined by 3H-thymidine incorporation. Data from 9 healthy controls and 10 acromegalics are presented as mean ± SEM.

tosterone replacement therapy in men with idiopathic hypogonadotrophic hypogonadism has been reported to increase the percentage of CD16+ cells and to diminish the percentage of CD4+ cells [26], whereas another study found no correlation between serum-free testosterone levels and the CD4/CD8 ratio in older, osteoporotic men [27]. However, in our 3 male patients, testosterone levels were in the normal range and therefore no influence of sex steroids on NK cells could be expected anyway.

GH appears also to exert no effect on serum immunoglobulin concentrations and lymphocyte cell surface marker distribution, since the acromegalics under study
exhibited no abnormalities. This is further supported by similar findings of a previous study [13] by a lack of effect of GH deficiency on these parameters [6, 25, 28] and is in accordance with the observed normal frequency of infections in acromegalic patients. The unchanged metabolic burst activity described here can be interpreted in the same way.

An increase in \( \text{O}_2\) production and a concomitant increase in phagocytic activity were reported when alveolar porcine macrophages were incubated with various concentrations of recombinant porcine GH in vitro [11]. This effect was similar to the one induced by IFN-\(\gamma\), the classical stimulus of macrophages [11]. Furthermore, Edwards et al. [29] demonstrated an enhanced phagocytic activity of monocytes toward opsonized \textit{Listeria monocytogenes} in vitro. In the present study we also found an increased phagocytic activity of cells in acromegaly, which indicates that GH, as a newly defined activating factor in vitro, can also increase the potency of macrophages to kill bacteria in patients with permanently elevated GH levels. Similarly, GH added to granulocytes in vitro leads to an increase in metabolic burst activity, which was also observed in vivo [11, 30]. These studies reported that GH increases the activities of two key enzymes in granulocytes and the amount of free radicals in supernatants of cultured cells. In contrast, we could not observe any difference in the number of free radicals producing granulocytes in acromegalic patients and healthy controls. So one explanation could be that GH increases the activity of key enzymes in granulocytes, but does not affect the number of cells inducing oxidative burst after stimulation.

Persistent elevation of GH for many years appears not to affect the percentage of lymphocytes, especially B cells, but to induce a higher expression of CD71 on CD4+ and CD8+ cells indicating an enhanced activity of T cells in the acromegalic subjects. From our data we cannot decide whether this is a direct action of GH or an indirect effect mediated through IGF-I.

Controversial data about the effect of GH on the lymphoproliferative response to PHA can be found in the literature [28, 31–33]. In the present study, the lymphocyte transformation response to PHA at higher concentrations tended to be elevated, but the difference failed to be significant.

Long-lasting GH elevation appears to induce only marginal stimulatory effects on the immune system, whereby most of the previously described effects of GH are not observed. Continuous elevation of plasma GH concentrations with occupation of nearly all receptors could thus hamper the biological activity of GH on the immune system. These findings are comparable in some points to those observed in hyperprolactinemic patients, where the previously reported acute effects of PRL on the immune system were also abolished [34].

Acknowledgment

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**References**


